Criteria Specification

ClinGen InSiGHT Hereditary Colorectal Cancer/Polyposis Expert Panel Specifications to the ACMG/AMP Variant Interpretation Guidelines for APC Version 1.0.0

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Description : The following criteria are for classic or attenuated familial adenomatous polyposis only and does not apply to Gastric adenocarcinoma and proximal polyposis of the stomach (GAPPS, MONDO:0017790). The preferred transcript for coding, intronic and promoter 1A variants is NM_000038.6 (MANE transcript). The NM_001127510.2 transcript differs from NM_000038.6 in the number of "non-coding" exons in the 5' region, which results in different exon numbering (in NM_000038.6 there is only one non-coding exon, in NM_001127510.2 there is one additional non-coding exon and one non-coding exon overlapping with NM_000038.6; the 15 coding exons are the same). For the promoter 1B deletion the preferred transcript is NM_001127511.3, which has an alternative coding exon 1. The LRG_130 summarizes all three “additional” exons of the previously mentioned transcripts, resulting in 18 exons). To standardize, variants in this document are described in HGVS nomenclature according to their positions in the NM_000038.6 transcript unless otherwise specified. Numbered exons in this document refers to exons 1-16 in the NM_000038.6transcript. Refer to Supplementary Table 1 for exon number conversions. It is important to note that these criteria are not developed for low/moderate penetrant variants (e. g. c.3920T>A p.(Ile1307Lys) and c.3949G>C p.(Glu1317Gln)).

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Rules for APC

Gene: APC (HGNC:583)

HGNC Name: APC regulator of WNT signaling pathway

Preferred Transcript: NM_000038.6

Criteria & Strength Specifications

PVS1

Original ACMG Summary

Null variant (nonsense, frameshift, canonical +/−1 or 2 splice sites, initiation codon, single or multi-exon deletion) in a gene where loss of function (LOF) is a known mechanism of disease.

Caveats:

- Beware of genes where LOF is not a known disease mechanism (e.g. GFAP, MYH7).
- Use caution interpreting LOF variants at the extreme 3’ end of a gene.
- Use caution with splice variants that are predicted to lead to exon skipping but leave the remainder of the protein intact.
- Use caution in the presence of multiple transcripts.

Very Strong

Null variant in a gene where LOF is a known mechanism of disease. As per modified decision tree (**Figure 1**) [Reference 1].

Modification Type: Gene-specific,Strength

Strong

Null variant in a gene where LOF is a known mechanism of disease. As per modified decision tree (**Figure 1**) [Reference 1].

Modification Type: Gene-specific,Strength

Moderate

Null variant in a gene where LOF is a known mechanism of disease. As per modified decision tree (**Figure 1**) [Reference 1].

Modification Type: Gene-specific,Strength

Supporting

Null variant in a gene where LOF is a known mechanism of disease. As per modified decision tree (**Figure 1**) [Reference 1].

Modification Type: Gene-specific,Strength

PS1

Original ACMG Summary

Same amino acid change as a previously established pathogenic variant regardless of nucleotide change.
Example: Val->Leu caused by either G>C or G>T in the same codon.
Caveat: Beware of changes that impact splicing rather than at the amino acid/protein level.

Strong

The previously established variant was classified as Pathogenic according to the _APC_-specific modifications.

This criterion can be applied to both missense and splice variants in *APC*.

Missense variants: when the variant under assessment results in the same amino acid change as previously established (Likely) Pathogenic variant(s).

There are currently only two Likely Pathogenic missense variants: c.3077A>G p.(Asn1026Ser) and c.3084T>A p.(Ser1028Arg). Other variants leading to the same

missense change at these positions meet PS1_Moderate. No missense variant has been classified as Pathogenic based on current evidence.

Splice variants: when the variant under assessment affects splicing at the same nucleotide as a previously established (Likely) Pathogenic variant. The splice prediction must be above defined thresholds [1] or similar to the previously established variant by multiple *in silico* predictors.

[1] See Supplemental material - Evaluation of canonical ±1 or 2 splice sites

Modification Type: Gene-specific,Strength

Moderate

The previously established variant was classified as Likely Pathogenic according to the _APC_-specific modifications.

This criterion can be applied to both missense and splice variants in *APC*.

Missense variants: when the variant under assessment results in the same amino acid change as previously established (Likely) Pathogenic variant(s).

There are currently only two Likely Pathogenic missense variants: c.3077A>G p.(Asn1026Ser) and c.3084T>A p.(Ser1028Arg). Other variants leading to the same missense change at these positions meet PS1_Moderate. No missense variant has been classified as Pathogenic based on current evidence.

Splice variants: when the variant under assessment affects splicing at the same nucleotide as a previously established (Likely) Pathogenic variant. The splice prediction must be above defined thresholds [1] or similar to the previously established variant by multiple *in silico* predictors.

[1] See Supplemental material - Evaluation of canonical ±1 or 2 splice sites

Modification Type: Gene-specific,Strength

PS2

Original ACMG Summary

De novo (both maternity and paternity confirmed) in a patient with the disease and no family history.
Note: Confirmation of paternity only is insufficient. Egg donation, surrogate motherhood, errors in embryo transfer, etc. can contribute to non-maternity.

Very Strong

≥ 4 *de novo* scores. For curation of *de novo* score see **Tables 1** and **2**.

Modification Type: Gene-specific,Strength

Strong

2-3 *de novo* scores. For curation of *de novo* score see **Tables 1** and **2**.

Modification Type: Gene-specific,Strength

Moderate

1 *de novo* score. For curation of *de novo* score see **Tables 1** and **2**.

Modification Type: Gene-specific,Strength

PS3

Original ACMG Summary

Well-established in vitro or in vivo functional studies supportive of a damaging effect on the gene or gene product.
Note: Functional studies that have been validated and shown to be reproducible and robust in a clinical diagnostic laboratory setting are considered the most well-established.

Very Strong

RNA assays show

1. a premature stop codon
   OR
2. inframe skipping of exon 13 or 14

AND the absence of full-length transcript.

Modification Type: Gene-specific,Strength

Strong

RNA assays show

1. a premature stop codon
   OR
2. inframe skipping of exon 13 or 14

AND < 10% of full-length transcript.

Modification Type: Gene-specific,Strength

Moderate

RNA assays show

1. a premature stop codon
   OR
2. inframe skipping of exon 13 or 14
   OR
3. other inframe skipping AND absent or < 10% full-length transcript.

Modification Type: Gene-specific,Strength

Supporting

RNA assays show

1. inframe skipping of of exons other than exon 13 or 14
   OR
2. over-expression of an alternative transcript

Protein assays show

Increased β-catenin regulated transcription activity and/or decreased binding to β-catenin by surface plasmon resonance (only for variants within the β-catenin binding domain, which refers to codons 959-2129 of *APC*) [Reference 2].

Modification Type: Gene-specific,Strength

PS4

Original ACMG Summary

The prevalence of the variant in affected individuals is significantly increased compared to the prevalence in controls.
Note 1: Relative risk (RR) or odds ratio (OR), as obtained from case-control studies, is >5.0 and the confidence interval around the estimate of RR or OR does not include 1.0. See manuscript for detailed guidance.
Note 2: In instances of very rare variants where case-control studies may not reach statistical significance, the prior observation of the variant in multiple unrelated patients with the same phenotype, and its absence in controls, may be used as moderate level of evidence.

Very Strong

≥ 16 phenotype points. For phenotype points curation see **Table 1**.

Modification Type: Gene-specific,Strength

Strong

4-15 phenotype points. For phenotype points curation see **Table 1**.

Modification Gene-specific,Strength

Type:

Moderate

2-3 phenotype points. For phenotype points curation see **Table 1**.

Modification Gene-specific,Strength
Type:

Supporting

1 phenotype point. For phenotype points curation see **Table 1**.

Modification Gene-specific,Strength
Type:

PM1

Original ACMG Summary

Located in a mutational hot spot and/or critical and well-established functional domain (e.g. active site of an enzyme) without benign variation.

Not Applicable

PM2

Original ACMG Summary

Absent from controls (or at extremely low frequency if recessive) in Exome Sequencing Project, 1000 Genomes or Exome Aggregation Consortium.
Caveat: Population data for indels may be poorly called by next generation sequencing.

Supporting

Rare in controls is defined by an allele frequency ≤ 0.0003% (0.000003).

Modification Gene-specific,Strength
Type:

PM3

Original ACMG Summary

For recessive disorders, detected in trans with a pathogenic variant
Note: This requires testing of parents (or offspring) to determine phase.

Not Applicable

PM4

Original ACMG Summary

Protein length changes due to in-frame deletions/insertions in a non-repeat region or stop-loss variants.

Not Applicable

PM5

Original ACMG Summary

Novel missense change at an amino acid residue where a different missense change determined to be pathogenic has been seen before.
Example: Arg156His is pathogenic; now you observe Arg156Cys.
Caveat: Beware of changes that impact splicing rather than at the amino acid/protein level.

Moderate

The reported missense variant was determined to be Pathogenic according to the _APC_-specific modifications.

There are currently only two Likely Pathogenic missense variants: c.3077A>G p.(Asn1026Ser) and c.3084T>A p.(Ser1028Arg). Other different missense variants at these positions meet PM5_supporting. No missense variant has been classified as Pathogenic based on current evidence.

Grantham´s distance of the variant under assessment must have an equal or higher score than the reported variant [Reference 3].

Modification Type: Gene-specific,Strength

Supporting

The reported missense variant was determined to be Likely Pathogenic according to the _APC_-specific modifications.

There are currently only two Likely Pathogenic missense variants: c.3077A>G p.(Asn1026Ser) and c.3084T>A p.(Ser1028Arg). Other different missense variants at these positions meet PM5_supporting. No missense variant has been classified as Pathogenic based on current evidence.

Grantham´s distance of the variant under assessment must have an equal or higher score than the reported variant [Reference 3].

Modification Type: Gene-specific,Strength

PM6

Original ACMG Summary

Assumed de novo, but without confirmation of paternity and maternity.

Strong

2-3 *de novo* scores. For curation of *de novo* score see **Tables 1** and **2**.

Modification Type: Gene-specific,Strength

Moderate

1 *de novo* scores. For curation of *de novo* score see **Tables 1** and **2**.

Modification Type: Gene-specific,Strength

Supporting

0.5 *de novo* scores. For curation of *de novo* score see **Tables 1** and **2**.

Modification Type: Gene-specific,Strength

Instructions: PM6_VeryStrong: ≥ 4 de novo scores. For curation of de novo score see Tables 1 and 2.

PP1

Original ACMG Summary

Co-segregation with disease in multiple affected family members in a gene definitively known to cause the disease.
Note: May be used as stronger evidence with increasing segregation data.

Strong

Variant segregates in ≥ 7 meioses in ≥ 2 families.

Modification Type: Strength

Moderate

Variant segregates in 5-6 meioses in ≥ 1 family.

Modification Type: Strength

Supporting

Variant segregates in 3-4 meioses in ≥ 1 familiy.

Modification Type: Strength

PP2

Original ACMG Summary

Missense variant in a gene that has a low rate of benign missense variation and where missense variants are a common mechanism of disease.

Not Applicable

PP3

Original ACMG Summary

Multiple lines of computational evidence support a deleterious effect on the gene or gene product (conservation, evolutionary, splicing impact, etc.).
Caveat: As many in silico algorithms use the same or very similar input for their predictions, each algorithm should not be counted as an independent criterion. PP3 can be used only once in any evaluation of a variant.

Supporting

This criterion can be applied to missense and non-canonical splicing variants.

Missense variants: Do not use computational prediction models for conservation, evolution, etc. *In silico* splicing predictors should be used for presumed missense variants to reveal possible splicing effects.

Non-canonical splicing variants: Multiple *in silico* splicing predictors support a deleterious effect.

Modification Type: Gene-specific,Strength

PP4

Original ACMG Summary

Patient’s phenotype or family history is highly specific for a disease with a single genetic etiology.

Not Applicable

PP5

Original ACMG Summary

Reputable source recently reports variant as pathogenic, but the evidence is not available to the laboratory to perform an independent evaluation.

Not Applicable

This criterion is not for use as recommended by the ClinGen Sequence Variant Interpretation VCEP Review Committee. PubMed : 29543229

BA1

Original ACMG Summary

Allele frequency is above 5% in Exome Sequencing Project, 1000 Genomes or Exome Aggregation Consortium.

Stand Alone

Allele frequency **≥ 0.1%** (0.001).

Modification Type: Gene-specific

BS1

Original ACMG Summary

Allele frequency is greater than expected for disorder.

Strong

Allele frequency **≥ 0.001%** (0.00001).

Modification Type: Gene-specific

BS2

Original ACMG Summary

Observed in a healthy adult individual for a recessive (homozygous), dominant (heterozygous), or X-linked (hemizygous) disorder, with full penetrance expected at an early age.

Strong

≥ 10 points for healthy individuals **OR** ≥ 2 times in homozygous state.

A **healthy individual** worth 1 point is defined by:

Age ≥ 50 years
+ Less than 5 adenomatous polyps in a colonoscopy
+ Absence of features in Table 1

OR

Age ≥ 50 years
+ Colorectal cancer/polyposis was not the indication for testing

A **healthy individual** worth 0.5 points is defined by keywords including control, non-cancer, normal, unaffected population.

Modification Type: Gene-specific,Strength

Supporting

≥ 3 points for healthy individuals.

A **healthy individual** worth 1 point is defined by:

Age ≥ 50 years
+ Less than 5 adenomatous polyps in a colonoscopy
+ Absence of features in Table 1

OR

Age ≥ 50 years
+ Colorectal cancer/polyposis was not the indication for testing

A **healthy individual** worth 0.5 points is defined by keywords including control, non-cancer, normal, unaffected population.

Modification Type: Gene-specific,Strength

BS3

Original ACMG Summary

Well-established in vitro or in vivo functional studies show no damaging effect on protein function or splicing.

Strong

RNA assay of a synonymous or intronic variant in constitutional patient sample demonstrates no mRNA aberration

AND

if biallelic expression is shown and/or nonsense-mediated decay inhibition was used.

Modification Type: Gene-specific,Strength

Supporting

RNA assay of a synonymous or intronic variant in constitutional patient sample demonstrates no mRNA aberration

OR

Protein assay show retention of β-catenin regulated transcription activity comparable to wild-type (only for variants within the β-catenin binding domain, which refers to codons 959-2129 of *APC*, see PMID: 33348689)

Modification Type: Gene-specific,Strength

BS4

Original ACMG Summary

Lack of segregation in affected members of a family.
Caveat: The presence of phenocopies for common phenotypes (i.e. cancer, epilepsy) can mimic lack of segregation among affected individuals. Also, families may have more than one pathogenic variant contributing to an autosomal dominant disorder, further confounding an apparent lack of segregation.

Strong

Affected member without the variant must score at least 1 phenotype point or at least two affected members without the variant must each score at least 0.5 phenotype points (see **Table 1**).

Modification Type: Gene-specific,Strength

Supporting

Affected member without the variant must score at least 0.5 phenotype points (see **Table 1**).

Modification Type: Gene-specific,Strength

BP1

Original ACMG Summary

Missense variant in a gene for which primarily truncating variants are known to cause disease.

Supporting

Exception: not applicable to missense variants located in the first 15-amino acid repeat of the β-catenin binding domain (codon 1021-1035) [Reference 3].

Modification No change
Type:

BP2

Original ACMG Summary

Observed in trans with a pathogenic variant for a fully penetrant dominant gene/disorder or observed in cis with a pathogenic variant in any inheritance pattern.

Supporting

Observed *in trans* with a (Likely) Pathogenic *APC* variant **OR** ≥ 3 times in an unknown phase with different (Likely) Pathogenic *APC* variants.

Modification Gene-specific
Type:

BP3

Original ACMG Summary

In frame-deletions/insertions in a repetitive region without a known function.

Not Applicable

BP4

Original ACMG Summary

Multiple lines of computational evidence suggest no impact on gene or gene product (conservation, evolutionary, splicing impact, etc)
Caveat: As many in silico algorithms use the same or very similar input for their predictions, each algorithm cannot be counted as an independent criterion. BP4 can be used only once in any evaluation of a variant.

Supporting

Missense variants: BP4 is not applicable.

Synonymous (silent) or intronic variants: Multiple in silico splicing predictors suggest no impact on gene or gene product.

Modification Gene-specific

Type:

BP5

Original ACMG Summary

Variant found in a case with an alternate molecular basis for disease.

Supporting

Only applicable for an alternate genetic basis of the colorectal polyposis phenotype.

Modification No change
Type:

BP6

Original ACMG Summary

Reputable source recently reports variant as benign, but the evidence is not available to the laboratory to perform an independent evaluation.

Not Applicable

This criterion is not for use as recommended by the ClinGen Sequence Variant Interpretation VCEP Review Committee. PubMed : 29543229

BP7

Original ACMG Summary

A synonymous variant for which splicing prediction algorithms predict no impact to the splice consensus sequence nor the creation of a new splice site AND the nucleotide is not highly conserved.

Supporting

A synonymous (silent) or intronic variant at or beyond +7/–21 for which multiple splicing prediction algorithms predict no impact to the splice consensus sequence nor the creation of a new splice site.

Modification General recommendation
Type:

Rules for Combining Criteria

Pathogenic

1 Very Strong *(PVS1, PS2_Very Strong, PS3_Very Strong, PS4_Very Strong)* **AND ≥ 1 Strong** *(PVS1_Strong, PS1, PS2, PS3, PS4, PM6_Strong, PP1_Strong)*

1 Very Strong *(PVS1, PS2_Very Strong, PS3_Very Strong, PS4_Very Strong)* **AND ≥ 2 Moderate** *(PVS1_Moderate, PS1_Moderate, PS2_Moderate, PS3_Moderate, PS4_Moderate, PM5, PM6, PP1_Moderate)*

1 Very Strong *(PVS1, PS2_Very Strong, PS3_Very Strong, PS4_Very Strong)* **AND 1 Moderate** *(PVS1_Moderate, PS1_Moderate, PS2_Moderate, PS3_Moderate, PS4_Moderate, PM5, PM6, PP1_Moderate)* **AND**

1 Supporting *(PVS1_Supporting, PS3_Supporting, PS4_Supporting, PM2_Supporting, PM5_Supporting, PM6_Supporting, PP1, PP3)*

1 Very Strong *(PVS1, PS2_Very Strong, PS3_Very Strong, PS4_Very Strong)* **AND ≥ 2 Supporting** *(PVS1_Supporting, PS3_Supporting, PS4_Supporting, PM2_Supporting, PM5_Supporting, PM6_Supporting, PP1, PP3)*

≥ 2 Strong *(PVS1_Strong, PS1, PS2, PS3, PS4, PM6_Strong, PP1_Strong)*

1 Strong *(PVS1_Strong, PS1, PS2, PS3, PS4, PM6_Strong, PP1_Strong)* **AND ≥ 3 Moderate** *(PVS1_Moderate, PS1_Moderate, PS2_Moderate, PS3_Moderate, PS4_Moderate, PM5, PM6, PP1_Moderate)*

1 Strong *(PVS1_Strong, PS1, PS2, PS3, PS4, PM6_Strong, PP1_Strong)* **AND 2 Moderate** *(PVS1_Moderate, PS1_Moderate, PS2_Moderate, PS3_Moderate, PS4_Moderate, PM5, PM6, PP1_Moderate)* **AND ≥ 2**

Supporting *(PVS1_Supporting, PS3_Supporting, PS4_Supporting, PM2_Supporting, PM5_Supporting, PM6_Supporting, PP1, PP3)*

1 Strong *(PVS1_Strong, PS1, PS2, PS3, PS4, PM6_Strong, PP1_Strong)* **AND 1 Moderate** *(PVS1_Moderate, PS1_Moderate, PS2_Moderate, PS3_Moderate, PS4_Moderate, PM5, PM6, PP1_Moderate)* **AND ≥ 4**

Supporting *(PVS1_Supporting, PS3_Supporting, PS4_Supporting, PM2_Supporting, PM5_Supporting, PM6_Supporting, PP1, PP3)*

Likely Pathogenic

1 Very Strong *(PVS1, PS2_Very Strong, PS3_Very Strong, PS4_Very Strong)* **AND 1 Moderate** *(PVS1_Moderate, PS1_Moderate, PS2_Moderate, PS3_Moderate, PS4_Moderate, PM5, PM6, PP1_Moderate)*

Benign

≥ 2 Strong *(BS1, BS2, BS3, BS4)*

1 Stand Alone *(BA1)*

Likely Benign

1 Strong *(BS1, BS2, BS3, BS4)* **AND 1 Supporting** *(BS2_Supporting, BS3_Supporting, BS4_Supporting, BP1, BP2, BP4, BP5, BP7)*

≥ 2 Supporting *(BS2_Supporting, BS3_Supporting, BS4_Supporting, BP1, BP2, BP4, BP5, BP7)*

1 Strong *(BS1, BS2, BS3, BS4)*

Files & Images

PVS1 decision tree: Modified decision tree for PVS1_Variable: Null variant in a gene where LOF is a known mechanism of disease [Reference 1].

Full criteria specification including all supplementary material: ClinGen InSiGHT Hereditary Colorectal Cancer/Polyposis Variant Curation Expert Panel Specifications to the ACMG/AMP Variant Interpretation Guidelines Version 1 for the APC gene

Table 1 & Table 2: Table 1: Point system for phenotypic description relevant to criteria PS2, PS4, PM6, PP1 and BS4; Table 2: Curation of de novo score for PS2 / PM6 based on the phenotype point system.

References

1. Abou Tayoun AN Pesaran T et al. *Recommendations for interpreting the loss of function PVS1 ACMG/AMP variant criterion.* **Hum Mutat** (2018) 39 (11) p. 1517-1524. 10.1002/humu.23626 30192042
2. Juanes MA *Cytoskeletal Control and Wnt Signaling-APC's Dual Contributions in Stem Cell Division and Colorectal Cancer.* **Cancers (Basel)** (2020) 12 (12) 10.3390/cancers12123811 33348689
3. Grantham R *Amino acid difference formula to help explain protein evolution.* **Science** (1974) 185 (4154) p. 862-4. 10.1126/science.185.4154.862 4843792